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pRmHa-3

In the NAR paper (Bunch et al, 1988 16:1043-1061), on page 1055, I made reference to expression vectors that contain additional unique cloning sites between the metallothionein promoter and the ADH polyadenylation signal. The construction of these plasmids is diagrammed in the following figures 1 and 2.

Figure 1 describes the construction of the expression vector pRmHa-2. *Eco*RI linkers were ligated onto the *Stu*I end of the *Eco*RI-*Stu*I metallothionein promoter fragment. The resulting fragment contains *Eco*RI restriction endonuclease sites at each end. This was cloned into the *Eco*RI site of pHa-1, which is described in the preceding paper. The resulting expression vector pRmHa-2 contains the additional unique cloning sites *Sac*I, *Kpn*I and *Sma*I that were not in pRmHa-1. As there are two *Eco*RI (E) sites, *Eco*RI does not serve as a unique cloning site.

*Eco*RI is perhaps the most common restriction endonuclease site used in cloning procedures. For this reason it is useful to modify pRmHa-2 so that it contains a unique *Eco*RI site following the metallothionein promoter. This is accomplished by the steps outlined in figure 2 and results in the expression vector pRmHa-3. In the first step, the *Eco*RI site that is 5', or upstream, of the metallothionein promoter in the vector pRmHa-1 was removed. This was accomplished by digesting pRmHa-1 with *Eco*RI, removing the single stranded ends by treating them with mung bean nuclease and then ligating the resulting molecules. This results in the removal of 4 base pairs from the *Eco*RI site. The *Sph*I fragment from the plasmid pRmHa-1 (-*Eco*RI) was then replaced by the *Sph*I fragment from pRmHa-2 which contains the additional cloning sites. This results in the expression vector pRmHa-3 which contains *Eco*RI, *Sac*I, *Kpn*I, *Sma*I, *Bam*HI, *Sal*I, *Hin*dI, and Acc1 as unique cloning sites following the metallothionein promoter.

~~**pH8C0**– Contains the bacterial DHFR gene and was constructed by Luey Cherbas. The reference is Kelly et al. 1991 Cell 67: 687-699.~~

Variable Effects of Copper Concentrations

In the NAR paper, we noted that 0.7 mM $CuSO_4$ gave close to maximal induction of the metallothionein promoter and did not alter the growth rate of the cells. In later experiments, I have noticed that 0.7 mM $CuSO_4$ did slow cell growth by as much as 50%. Preliminary experiments also suggested that 0.3 mM $CuSO_4$ was resulting in induction of the metallothionein promoter as efficiently as 0.7 Mm $CuSO_4$. The reason for these differences between the earlier and the later experiments is not clear. One possible explanation is that different batches of fetal calf serum, which is added to the growth medium, may contain factors that bind copper. This could result in different effective concentrations of copper in medium prepared with different batches of fetal calf serum. I have not investigated this further and in later experiments I have used 0.3 mM $CuSO_4$.

Figure 1. Construction of the Expression Vector pRmHa-2.

A detailed description of the construction of pRmHa-2 is provided in the Addendum to the Metallothionein Paper. The abbreviations are as follows: Mt, metallothionein; R, *Eco*RI; Ts, transcription start site; B, *Bam*HI; A, *Acd*; Sa, *Sal*; Hc, *Hind*I; A^+ polyadenylation/cleavage site; St, *Stu*; Ori, origin of replication and Amp^r, the beta-lactamase gene that confers resistance to ampicillin.

Figure 2. Construction of the Expression Vector pRmHa-3.

A detailed description of the construction of pRmHa-3 is provided in the Addendum to the Metallothionein Paper. The abbreviations are the same as those in figure 4-1 with the addition of Sp, *Spe*.

Fig 1

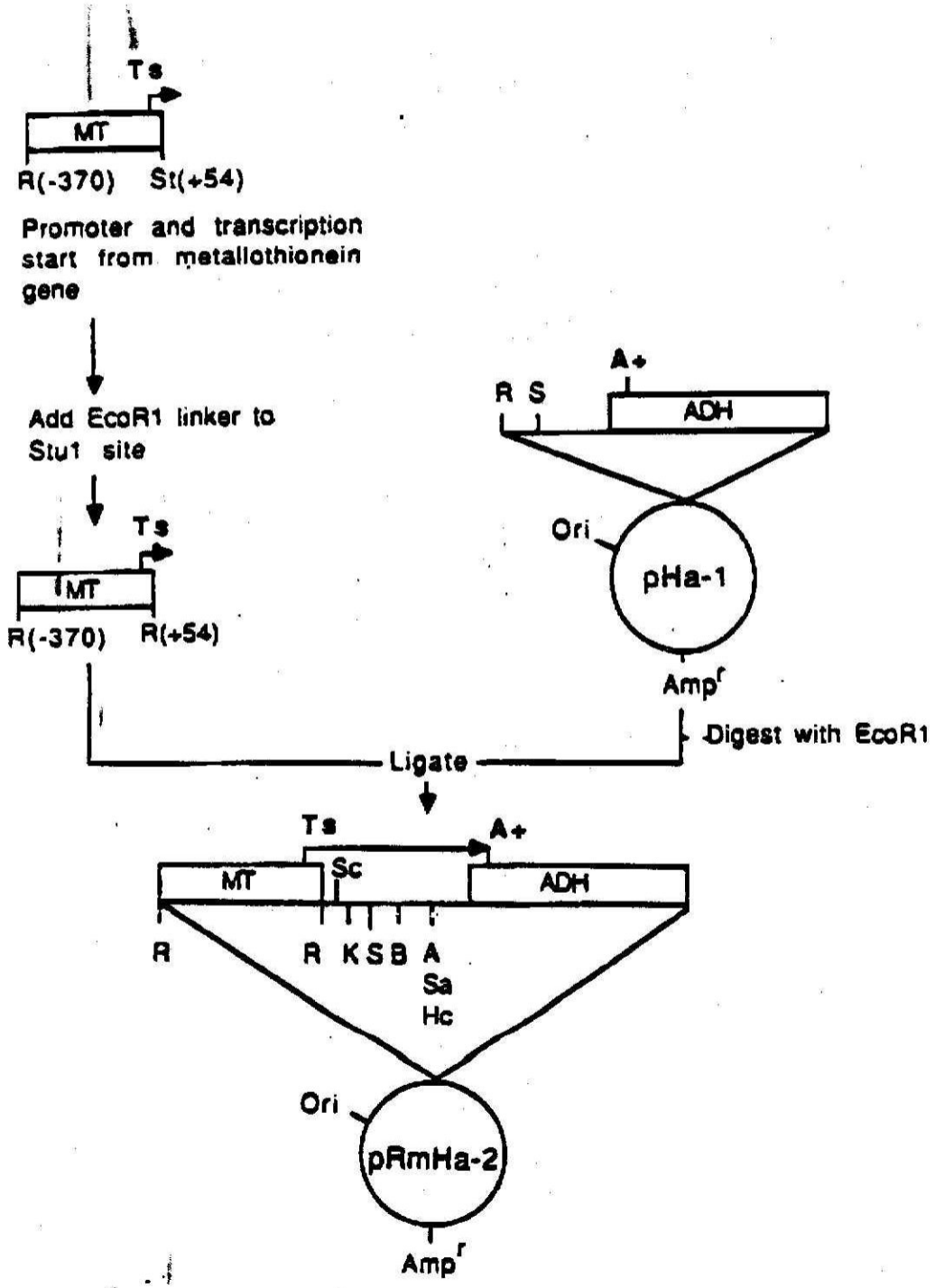
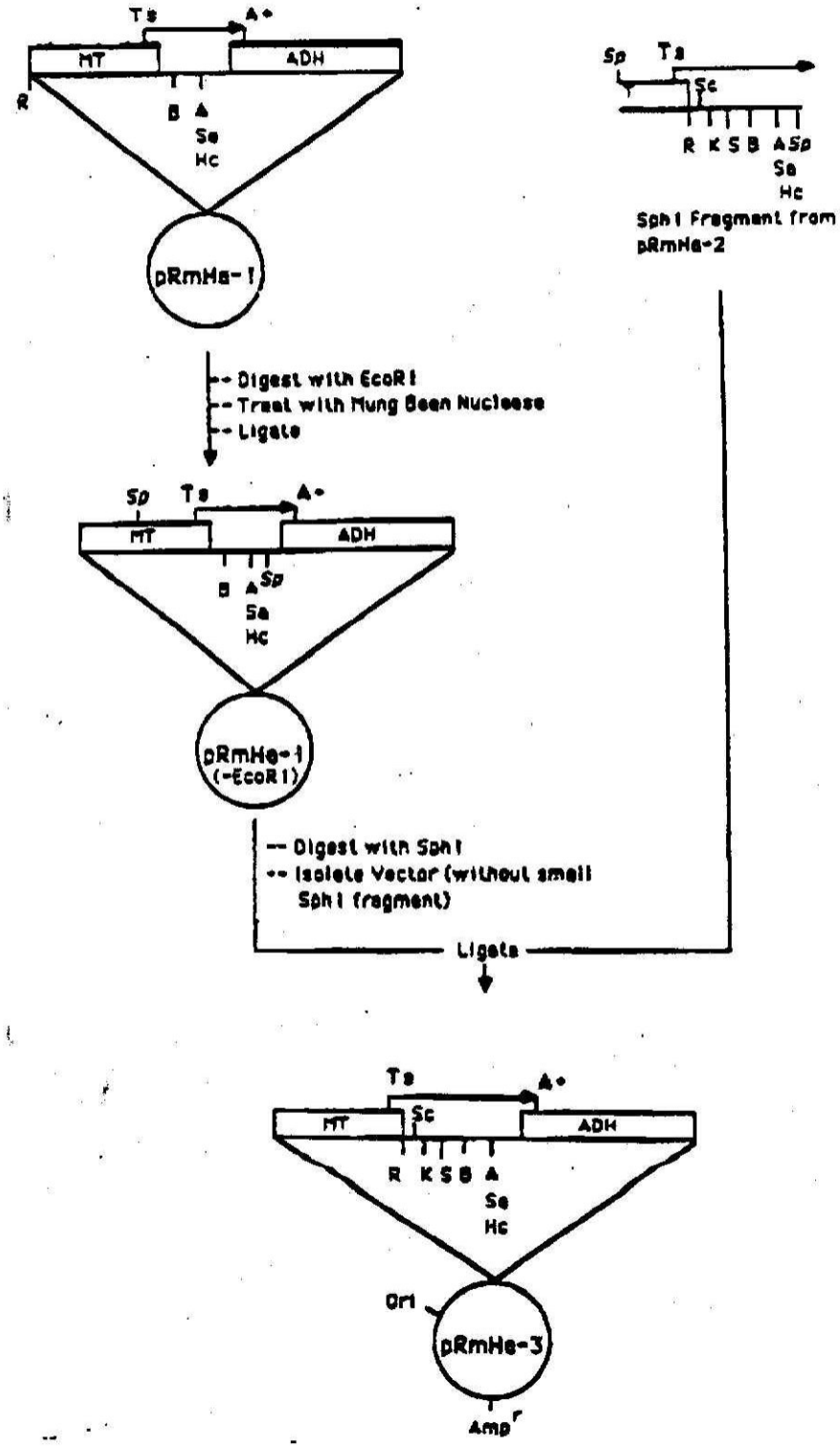

Ts
MT
R(-370) St(+54)
Promoter and transcription start from metallothionein gene
Add EcoR1 linker to Stu1 site
Ts
MT
R(-370) R(+54)
A+
R S
ADH
Ori
pHa-1
Amp^r
Digest with EcoR1
Ligate
Ts
A+
MT
Sc
ADH
R
R K S B A
Sa
Hc
Ori
pRmHa-2
Amp^r

Fig 2


Ts
A*
MT
ADH
R
B
A
Se
Hc
pRmHe-1
Sp
Ts
Sc
R K S B A Sp
Se
Hc
Sph I Fragment from
pRmHe-2
-- Digest with EcoR I
-- Treat with Mung Bean Nuclease
-- Ligate
Sp
Ts
A*
MT
ADH
B
A Sp
Se
Hc
pRmHe-1
(-EcoR I)
-- Digest with Sph I
-- Isolate Vector (without small
Sph I fragment)
Ligate
Ts
A*
MT
Sc
ADH
R K S B A
Se
Hc
Ori
pRmHe-3
Amp r